



Salinity stress evaluation on nutrient uptake and chlorophyll sugarcane genotypes

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Abstract

In order to study the effects of salinity levels of the irrigation water resulted from the sugarcane field drainage which contained mineral salt (NaCl), four salinity levels (0, 3, 6, 9 ds.m⁻¹) and sugarcane genotypes (C4, C5, C3, CP48-103, C2, CP57-614, CP69-1062) in a potted plant were examined. It was a factorial experiment in randomized complete block design with three replications which was carried out in research center of Amirkabir sugarcane industrial company located in 45 km south of Ahvaz in 2011. Results indicated that as the salinity stress increased, chlorophyll concentration relatively decreased in all genotypes, but the rate of decrease in CP69-1062, C4, C5 genotypes was less than other genotypes. Chlorine and sodium content in the leaves of genotypes tolerant of C4 and C5 decreased as the salinity increased which indicates the genetic ability of these genotypes in preventing the toxic ion of chlorine from entering the plant. CP69-1062 genotype in comparison to CP48-103 and CP57-614 genotypes has the lowest rate of sodium in leaves and owns genetic potential in preventing sodium ion from entering the plant.

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Introduction

Soil salinity is an increasing problem of agricultural soil which decreases the plants growth rate and crop production particularly in arid and semiarid areas. In agricultural land which requires constant irrigation, even though some of the salt goes down through washing, some stays in the soil due to evaporation and gradually the soil concentration around the root increases (Apse *et al*, 1999). Direct effects of salinity on the plant growth could be divided to three main groups:

A: Increase of osmotic potential of the soil solution which leads to the decrease of water uptake by the plant;

B: destruction of soil tissues which reduces permeability of water and air;

C: specific ion toxicity.

Management of soil fertility especially for N and K in salt-affected soils is essential because these nutrients are required in high amounts for good crop growth and high production (Noaman, 2004).

Soil salinity can indirectly affect the plant growth by preventing biological processes such as nitrogen and nitrate production (Pessararakli *et al*, 1994). The increase of osmotic pressure reduces water and nutrients especially phosphorus, iron, manganese, zinc, and molybdenum. Plant growth is inhibited due to osmotic effects. Salinity stress affects nitrogen metabolism and protein synthesis and causes the accumulation of ammonium and nitrate ions as free amino acids (Sherivastava and srivastava, 2006).

Nitrogen is mainly involved in the initial growth processes, such as replication of chromosomes, synthesis of deoxyribonucleic acids and nuclear protein. Similarly, K⁺ is also involved in numerous physiological processes and both N and K⁺ influence plant growth in a synergistic way (Helal *et al*, 1975).

The yield losses of sugarcane in moderately salt-affected areas were calculated to be 62%. Different approaches are being used to utilize or to reclaim the salt-affected soils. Presently it is planned to utilize the moderately salt-affected soils for sugarcane production by the management of Na/K ratio (Pessararakli M, 1994).

The main reason of inhibiting growth by salinity is the problems in absorbing other minerals in competition with sodium. In low concentrations, sodium might really increase potassium intake, but in high concentrations it reduces the absorption of potassium. For example, the high concentrations of sodium in sugarcane and rice will decrease the absorption of potassium and the growth of two plants (Yazy Meybodi and Karabakh, 2002). It seems like that due to salinity most of the absorbed sodium accumulates in the leaf lamina where photosynthesis and other metabolic activities are done. These processes are incompatible with saline environment and there is a linear relationship between the rate of leaf sodium and the decrease of net photosynthesis (Plaut *et al*, 2007). Soltani Hoveize and Mirmohammadi Meybodi (2007) showed that as the salinity level increased more chloride and sodium were absorbed and transmitted and based on the cultivar different ratio of sodium salt was absorbed by potassium and calcium. Robinson in 1996 showed that in NCO.310 cultivar, as the electric conductivity of soil increased from 2.5 to 4.2 ds.m⁻¹ its germination and yield decreased up to 50% which indicates its sensitivity to salinity. CP69-1062 has a relatively good tolerance of soil salinity and in addition to proper yield in Khuzestan weather conditions, it has high rationing capability (Ehyai and Behbehani, 1992). CO1148 cultivar is a sugarcane cultivar which is half tolerant of salinity and a mainly commercial product which is cultivated in a large area of cane-prone regions of the world particularly in countries such as Pakistan which are facing salinity problem (Sandra, 2009). Shomili (2002) showed in his research that CP69-1062 cultivar has the ability to produce shoots properly in salinity conditions and produce dry

matter and distribute the roots reasonably in such conditions. Moreover, in CP69-1062 and CO1148 cultivars as the salinity increased, the ratio of chloride and sodium (sensitivity biochemical factors) to potassium (tolerance biochemical factor) was in a good condition which indicates the presence of tolerance factors in such cultivars. On the other hand, keeping a high level of nitrogen, phosphorus, and calcium in the roots and stems of these two cultivars is closely related to their tolerance of salinity. The stable rate of these elements in plant tissues could be used as an indicator of sugarcane tolerance of salinity resulting from NaCl. Plot *et al* (2007) observed that in the sugarcane which is exposed to salinity chloride and sodium (not potassium) accumulated more in the leaves and stems. Accumulation of chloride and sodium in LTM leaf in resistant H69-8235 was more than in sensitive H65-7052. As the concentration of sodium chloride in irrigation water increased, the absorption of sodium and chloride increased, too and most of it accumulated in the leaf lamina. At low concentration (1 mM sodium chloride) chloride uptake and accumulations was more than that of sodium, but at high concentrations sodium uptake and accumulation was more (Ranis, 2005). This research was carried out to achieve the following objectives:

- A: identifying the most resistant and the most sensitive cultivars of sugarcane to salinity stress.
- B: evaluating available commercial cultivars in relation to salinity cultivars.
- C: identifying salinity tolerant cultivars of sugarcane.

The present study was conducted to evaluate Nutrient uptake and chlorophyll content for obtaining economical sugarcane yield under saline conditions.

Material and methods

This research was carried out in 2011 in one of the greenhouses of the institute of research and development training of sugarcane located in 45th Km

Ahvaz-Khoramshahr road. The soil had Clay Loam tissue. Physical and chemical properties of the soil are shown in tables 1 and 2. Sugarcane cuttings were selected from CP57-614, CP69-1062, CP48- 103, C2, C3, C4 varieties. Then the cuttings were placed in plastic pots in the greenhouse until they sprouted. The height of each pot was 60 cm and the upper diameter was 39 cm, the volume was 25 liters and it was made of polyethylene and each one was filled with the field soil and a layer of coarse sand for drainage. The weight of dry soil in each pot was 66 kg. In this research the effects of salinity on morphological traits of 7 genotypes (C5, C4, C3, C2, CP48-103, CP57-614, CP69-106) and control concentrations of 3, 6, 9% sodium salt in greenhouse cultivation environment. It was a factorial experiment in randomized complete block design with three replications. 4 levels of water salinity including EC=1(S1), EC=3(S2), EC=6(S3), EC=9(S4) ds.m⁻¹ were applied after the plant establishment since November 22, 2011. EC was set by EC detector ZW-124. The rate of chlorophyll was measured by SPAD. To do so, the upper widely open leaves of the seedling were used. Dry matter of the shoots and the roots was separately ground by an electric grinder separately and then the powder was sifted by a 0.5 mm sieve in order to measure the ionic elements of potassium, sodium, chloride, nitrogen, phosphorus, and calcium. In order to examine the effects of salinity on nutrient uptake by different sugarcane cultivars the concentration of leaf nutrients was measured. In this regard, the rate of nitrogen (N) was measured by kjeldahl method, Na⁺ and K⁺ were measured by film photometer, P was measured by spectrophotometer, CL by silver nitrate titration method, and Ca⁺⁺ was measured by EDTA titration.

Table 1. Soil physical properties.

Sp (%)	Ec (ds/m)	pH	Sandy	Silt	Clay	Soil Texture
34	2/75	7/74	32/4	35/7	27/4	Sandy Loam

Table 2. Soil chemical properties.

Ca (Meq.l ⁻¹)	Mg (Meq.l ⁻¹)	Potassium solution (Meq.l ⁻¹)	Absorption Potassium (Meq.l ⁻¹)	Phosphor (Mg.kg ⁻¹)	Nitrogen %
9.12 Bicarbonate (Meq.l ⁻¹)	6.90 Sulfate. (Meq.l ⁻¹)	0.45 Sodium (Meq.l ⁻¹)	3.78 Chlorine (Meq.l ⁻¹)	4.35 Bicarbonate (Meq.l ⁻¹)	0.104 Sulfate (Meq.l ⁻¹)
6.22	12.17	13.28	29	6.22	12.17

Results and discussion

Chlorophyll Index

The results of table (3) show the significant effect of salinity and genotypes on chlorophyll index at 1% probability level. As the salinity increases the rate of photosynthesis decreases and the leaves get yellow. There is an inverse relationship between chlorophyll concentration of the leaf and different levels of salinity, and less reduction was observed in resistant genotypes. The results of the research are consistent with the findings of Singh *et al.* (2009). Diagram 1 shows the effects of salinity and genotype on the rate of chlorophyll. The highest rate of chlorophyll belongs to control treatment (EC₀) and C4 and C5 genotypes. Among the commercial and other genotypes, C4 has the highest rate of chlorophyll in the highest level of salinity. Kaya *et al.* (2006) reported that the chlorophyll content and photosynthesis rate of

sensitive varieties would decrease under salinity stress. The leaf chlorophyll index decreased as the sodium chloride concentration increased. There was a significant difference between the means of salinity and genotype at 1% probability level. As the levels of salinity stress increased, the concentration of chlorophyll decreased (Table 3). Kaya *et al.* (2006) reported that chlorophyll concentration is considered as an index for evaluating the source power because the leaves chlorophyll concentration is a key factor in determining the rate of photosynthesis and production of dry matter. Sing *et al.* (2009) reported that as the salinity levels increased the rate of the leaf chlorophyll decreased. As the stress density increased the intensity of green color of the leaves decreased and the number of chlorophyll decreased, too.

Table 3. Summary results of analysis variance of traits.

S.O.V	df	Chlorophyll Index	Ca	P	K	N	Na	Cl
R	2	13.65**	2.138**	0.001 **	16.512**	1.045*	1.155**	0.596**
Salinity	3	333.91**	22.311**	0.350*	333.514 **	0.350*	79.917*	8.164 **
Genotype	6	129.27**	1.571**	0.0001 ns	138.080**	0.387*	2.484**	1.855**
Salinity*Genotype	18	4.750**	0.103 ^{ns}	0.0001 ns	10.594**	0.007 ^{ns}	1.056 ns	0.313**
TE	54	1.951	0.100	0.0001	1.808	0.116	0.723	0.035
CV	-	5.56	14.12	8.24	5.24	16.27	14.14	3.93

*, ** ns : Significant at the 5 and 1 percent levels, respectively, and no significant difference

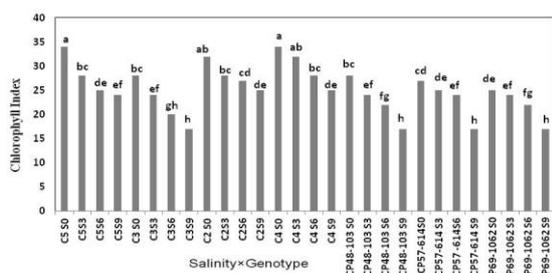


Diagram 1. Interaction effect of genotype and salinity on chlorophyll index.

Ionic Indices

Leaf Nitrogen

The results of Table (3) show that the effect of salinity stress on this trait was significant at 5% probability level, but the effect of genotype was not significant. Probably, genotypes absorb the same amount of nitrogen. Salinity stress decreases nitrogen absorption in the plant. It seems like that the negative effect of salinity on nitrogen concentration in the

plant could be attributed to the antagonistic effect between nitrate and chloride. There was a significant difference between the increase of salinity and the decrease of the leaf nitrogen. In tolerant genotypes this trend decreases. According to Ram *et al.* (1999), salinity decreases nitrogen in the plant which is due to its negative effect on nitrogen absorption and consumption. Diagram 2 shows the effects of salinity and genotype on the rate of leaf nitrogen. The highest rate of leaf nitrogen belongs to the control treatment (EC₀) and genotypes C4 and C5. As salinity increases the rate of leaf nitrogen decreases because the plant absorbs toxic elements such as sodium and chloride and prevents nitrogen uptake and causes the decrease of organs growth. In high level of salinity, C5 genotype had higher amount of nitrogen than other genotypes. It seems like that some of genetic characteristics of this genotype include less absorption of toxic ions and more uptake of nutrients. Salinity has a negative decreasing effect on nutrients absorption. The rate of leaf nitrogen in genotypes C2, C3, C4, at all salinity levels had almost the same stable trend, while in genotypes CP48-103, CP57-614, and CP69-1062 at salinity level of 9 ds.m⁻¹ the highest rate of leaf nitrogen belonged to CP69-1062. Genotypes CP48-103 and CP57-614 had the lowest rate of leaf nitrogen at the highest level of salinity. The signs of nitrogen shortage are usually the decrease of growth of some organs, and older leaves get narrow and yellow. Malakuti (1996). According to Baibordi (2010) salinity decreases the plant nitrogen which results from the negative effect of salinity on nitrogen uptake. Salinity decreases the nitrogen of the leaf and increases the entrance of toxic elements such as sodium and chloride to the plant.

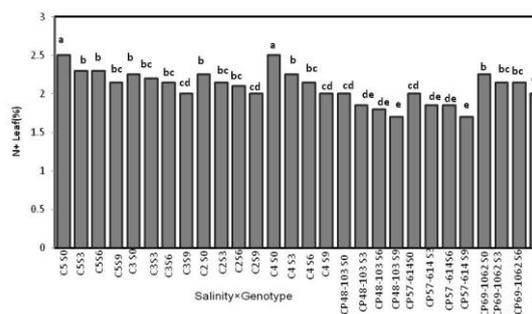


Diagram 2. Interaction effect of genotype and salinity on N⁺ leaf.

Leaf Potassium

Potassium is an important nutrient which is absorbed by sugar cane more than other nutrients. The results of table (3) show that the effect of salinity and genotype on the leaf potassium is significant at 1% probability level. As the salinity levels increase, the tolerant genotypes have the highest rate of the leaf potassium. It seems like that potassium modulates the toxic effects of chloride and sodium ions and thus tolerant genotypes are able to grow better in saline conditions. There was a significant difference between the content of potassium and sugarcane genotypes experimented in salinity conditions. Akhtar *et al.* (2003) reported a significant difference between the ratio of potassium in different genotypes of sugarcane at 1% probability level. They stated that even though this ratio decreases as the salinity increases, tolerant genotypes have a higher ratio of potassium. Diagram (3) shows that as the salinity increased the rate of leaf potassium decreased which was possibly due to the increase of sodium content which interferes in most species which are exposed to salinity stress and there is a negative relationship between sodium and potassium. The highest rate of leaf potassium was observed in the control treatment (EC₀) and C4 and C5 genotypes. C4 genotype had the highest rate of potassium at high levels of salinity in comparison to other genotypes. Probably, tolerant genotypes decrease the toxic effects of sodium and chloride ions. The highest rate of potassium at salinity level of 9 ds.m⁻¹ was observed in Cp69-1062 among CO48, CP57, CP69-1062 genotypes which are due to its

genetic characteristic, so that it absorbs more potassium in high concentrations of salt. There was no significant difference between CP48 and CP57 genotypes. Akhtar *et al* (1997) reported that as the salinity increased the rate of leaf potassium decreased which was due to the increase of sodium and the negative relationship between sodium and potassium. By increasing the salinity from 1 to 9 ds.m⁻¹ the rate of leaf potassium decreased from 26 to 18 mg/g because as the salinity increases potassium decreases. However, tolerant genotypes have more potassium at high levels of salinity.

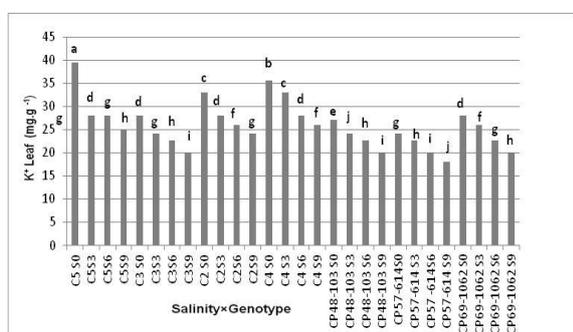


Diagram 3. Interaction effect of genotype and salinity on K⁺ leaf.

Leaf Calcium

The results of table (3) show that the effect of salinity and genotype on the leaf calcium is significant at 1% level. As the salinity level increased, the leaf calcium decreased. It seems like that toxic elements such as sodium and chloride increase and the rate of calcium decreases. Calcium plays an important role in regulating cell membrane processes and is absorbed by plants especially sugarcane in saline conditions. Therefore the increase of this element can be used as an index for tolerating salinity. Wahid *et al.* (1997) reported that there was a significant difference between the lines and levels of salinity in terms of calcium content. Diagram (4) shows that the highest rate of leaf calcium was observed in the control treatment (EC₀) and C5 genotype. C5 genotype had the highest rate of calcium at high salinity level in comparison to other genotypes because tolerant genotypes have more calcium ion than sensitive genotypes which is probably due to genetic

superiority of such genotypes. At salinity level of 9 ds.m⁻¹ the lowest rate of calcium was observed in CP57-614 and CP48-103 among CP69-1062, CP57-614, and CP48-103 genotypes because less absorption of calcium and potassium leads to absorption of toxic elements by plant which finally results in the decrease of plant growth. The highest rate of calcium was observed in CP69-1062 genotype. Probably, this genotype's tolerance of salinity is more than that of two other genotypes. Akhtar *et al.* (1997) reported that there was a significant difference between genotypes in terms of the content of calcium in the leaf and root. They observed that the rate of calcium reduction in tolerant genotype was less than sensitive genotype and thus concluded that having more content of calcium and potassium could modulate the toxicity of sodium and calcium ions and enable the tolerant genotype to grow and produce more in saline conditions. Wahid *et al.* (1997) reported that there was a significant difference in terms of calcium content between the lines and levels of salinity and the interactive effect of line on salinity.

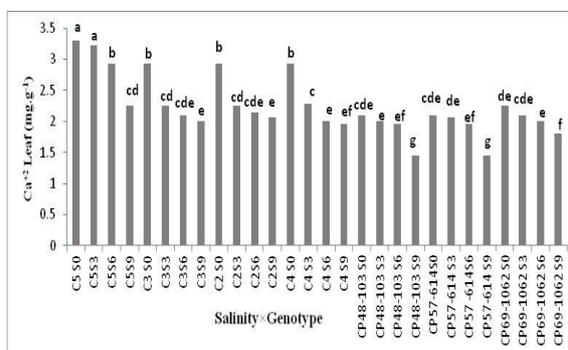


Diagram 4. Interaction effect of genotype and salinity on Ca⁺⁺ leaf.

Leaf Phosphor

The results of table (3) show that salinity is significant at 5% level. As the salinity increases, the rate of leaf phosphor decreases. Probably, the increase of chloride causes the decrease of phosphor because their uptake mechanism is the same. Phosphor is a vital element for the plant. Lack of phosphor slows down the growth rate and decreases the yield. Results show that genotype is not significant which is possibly

due to the fact that all genotypes absorb the same rate of phosphor. Roberts *et al* (1984) reported that the increase of salinity causes the decrease of phosphor uptake by the plant. Diagram (5) shows that the highest rate of leaf phosphor was observed in control treatment (EC₀) and C4 genotype. As the salinity increases, the rate of phosphor decreases. In salinity stress, the decrease of the release of phosphor from the soil to the surface of the root is possibly more than that of other nutrients because phosphor ion is attached to clay particles and is less available for the plan root and aerial organs. C4 genotype had the highest rate of phosphor in comparison to other genotypes at high salinity level because such tolerant genotypes of sugarcane well tolerate the changes caused by the environment salinity due to their genetic characteristic with minimal casualties. The highest rate of phosphor at salinity level of 9 ds.m⁻¹ among CP48-103, CP69-1062, C2, C3, and CP57-614 was observed in CP69-1062 and C2 genotypes. It seems like that they absorb less chloride ion. Gibson (1988) stated that phosphor is an important nutrient which directly affects the plant growth and development and as the salinity increases, its absorption by varieties which are sensitive to salinity decreases. Khalil *et al* (1967) reported that as the salinity of soil increases, the plant ability to access phosphor decreases because phosphor and chloride are both anions and both probably have the same absorption mechanism and the increase of chloride concentration due to its competition with phosphate ion has harmful effects on the absorption of phosphor. As salinity increases, the concentration of leaf phosphor decreases.

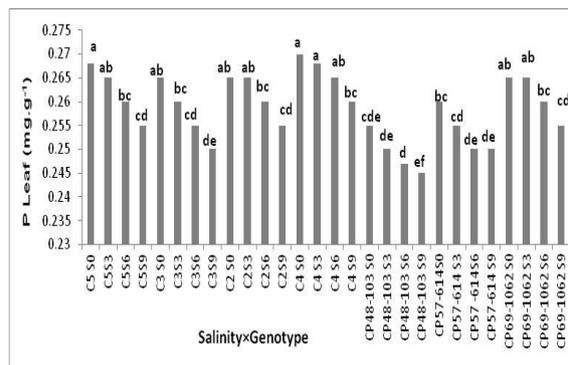


Diagram 5. Interaction effect of genotype and salinity on leaf phosphorus.

Leaf sodium

The results of table (3) show that salinity is significant for the components of the rate of leaf sodium and genotype at 1% level. Sodium is an element whose concentration in plant increases due to salinity. Tolerant genotypes at high salinity level absorb less sodium because the plant sends toxic ions toward the older leaves in order to save younger leaves from the harmful effects of such elements. In this research, there was a significant difference between the interactive effect of salinity and genotype. The significant interactive effect of genotype and salinity on the rate of leaf sodium probably indicates the little accumulation of this ion in tolerant genotype. The increase of sodium in plant due to salinity has been reported by many researchers including Baibordi (2010). Diagram 5 shows that the lowest rate of leaf sodium was observed in the control treatment (EC₀) and C4 genotype. At high level of salinity, C4 genotype had the lowest rate of sodium in comparison to other genotypes which is possibly due to its genetic potentiality to prevent sodium ion from entering the plant. At salinity level of 9 ds.m⁻¹, CP69-1062 has the lowest rate of leaf sodium among the commercial genotypes because there is an antagonistic relationship between sodium and potassium. As more potassium is absorbed, it prevents the absorption of sodium by the plant. There was not a remarkable difference between other genotypes at high salinity level. At highest level of salinity, the lowest rate of sodium is 1.7 mg/g and the highest rate of sodium is 2.15 mg.g⁻¹. the increase of sodium in plant due to

salinity has been reported by many researchers like Hirschi (2004). Baibordi (2010) reported that the decrease of sodium in resistant varieties was more than sensitive varieties to salinity. Akhtar *et al* (1977) reported that there was a significant difference between sugarcane genotypes in terms of sodium content of the leaf and root at 1% level. In their studies, they showed that in spite of the linear increase of sodium concentration through the increase of salinity, tolerant genotypes limited the entrance of sodium ion to the plant.

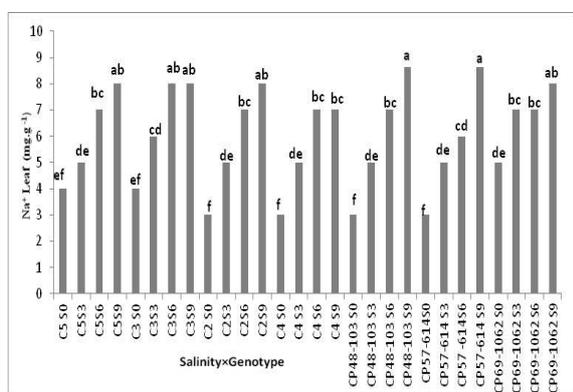


Diagram 6. Interaction effect of genotype and salinity on leaf Na⁺.

Leaf Chloride

The results of table (3) showed a significant difference between the effects of genotype and different levels of salinity on the leaf chloride at 1% level. As the salinity increased the chloride content in tolerant varieties had a decreasing trend which possibly indicates the ability of this genotype to prevent the toxic ion of chloride from entering the plant. Therefore, one of the probable mechanisms of sugarcane for avoiding the ion toxicity effects of salinity stress is to absorb less toxic chloride in tolerant varieties. Chodhury *et al* (1995) reported that there was a significant difference between sugarcane genotypes in terms of chloride content of area organs and the root. They stated that as the salinity increased, the concentration of chloride increased linearly. However, the increase in sensitive cultivar was more than the others. Diagram (6) shows that the lowest rate of leaf chloride belonged to the control treatment (EC₀) and C4 genotype. At high

salinity level, C4 and C5 genotypes had the lowest rate of chloride because they were possible able to prevent the toxic ion from entering the plant. Among CP69 - 1062, CP48-103, CP57-614, C3, C2 genotypes at high salinity level, CP69-1062 had the lowest rate of chloride. There was no significant difference between other genotypes. At highest salinity level, the lowest rate of chloride was 4 mg.g⁻¹ and the highest rate was 6 mg.g⁻¹. Robinson (1997) reported that in resistant cultivars, as the rate of salinity increased, the stable and gradual decrease of chloride and sodium and the increase of potassium were quite observable. This condition led to the modification and change of the ratio of potassium to sodium in these plants. Azevado and Taosa (2000) stated that as the salinity increased the concentration of chloride ion increased linearly. However, such an increase was more in sensitive genotype. Soltani Hoveize and Mirmohammadi Meybodi (2007) reported that genotypes which were resistant to salinity controlled the entrance of Cl⁻ ion to the plant and kept larger amounts of K and Ca ions in the plant.

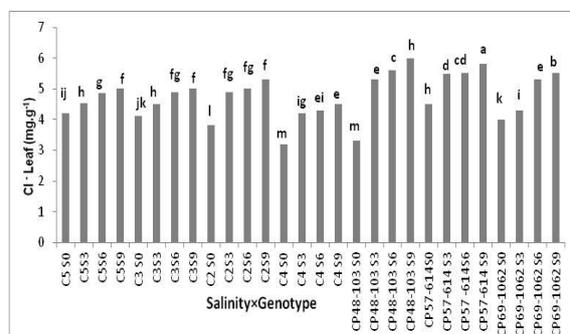


Diagram 7. Interaction effect of genotype and salinity on leaf Cl⁻.

Conclusion

Salinity has a negative effect on all ionic indices of sugarcane and the results showed that the increase of salinity stress more than 1 ds.m⁻¹ significantly decreased the chlorophyll concentration, but the reduction in C5 and C4 genotypes was less than other genotypes because these superior genotypes of sugarcane well tolerated the changes caused by the environment salinity due to their inherited genetic abilities and with minimal casualties. Among CP57-

614, CP48-103, CP69-1062, C2, C3 genotypes, Cp69-1062 is the most remarkable one whose physiological indices nearly decreased as the salinity level increased. As the salinity level increased, the rate of N, P, K, and Ca in C4 and C5 genotypes relatively decreased in comparison to other genotypes because they decrease the toxicity of chloride and sodium ions. C4 and C5 genotypes are probably superior to other genotypes since they prevent the harmful elements of chloride and sodium from entering the plant. Among CP57-614, CP48-103, CP69-1062, C2, C3 genotypes the best one is CP69-1062 which absorbs useful nutrients and prevents the harmful elements from entering the plant.

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